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Bt-transgenic Crops: just Another Pretty Insecticide or a Chance for a New Start in Resistance Management?*

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Abstract: Transgenic insect-resistant crops carrying genes from *Bacillus thuringiensis* were grown commercially for the first time in 1996 amid considerable public controversy about resistance management. Several resistance management strategies have been proposed for Bt-transgenic crops. The most promising with currently available technology is the use of refuges of non-transgenic crops, augmented where possible with high toxin expression in the plant and avoiding mosaics of different toxins and pesticides. One problem is that the refuge sizes that are seen as commercially and practically acceptable are generally too small to provide a comfortable margin for the delay of resistance. A promising long-term strategy for delaying resistance, and one which is more forgiving on refuge size, is the pyramiding of two or more insecticidal genes in the same plant. The critical limiting factor to resistance management for transgenic crops will be implementation, which will require cooperation among companies.

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1 INTRODUCTION

The crystal protein 'Cry endotoxins' produced by *Bacillus thuringiensis* (Bt) Berliner are among the most specific and potent insecticides known to man.[1] Unfortunately, because they must be eaten by the target insect and persist for only a few days after application, sprays produced with Bt toxins have had limited use.[2] It was realised more than a decade ago that problems with persistence, complete coverage of the plant (impossible with sprays, especially for the control of internal feeders like stem borers) and therefore ingestion could be overcome by transferring the *cry* genes to plants. Plants able to defend themselves effectively even against high densities of insects were produced by the early 1990s[3] and finally commercialised in the USA[1] and Australia in 1996. Bt-transgenic potatoes and maize were grown on only a very limited scale in 1996, but some 730 000 ha (1·8 million acres) of Bt transgenic cotton (about 13% of the crop) were planted in the USA,[1,4] with another 30 000 ha in Australia.

However, one of the very features that makes Bt-transgenic crops attractive, the increased persistence of the toxins (not to mention the increased efficacy that will encourage greater use of Bt toxins in this form), enhances the potential for evolution of resistance in the pests. This concern has probably evoked greater public and regulatory interest in resistance management than any other event in the history of pest control. Although not technically required by law, resistance management plans were a major issue in the registration process for both the US Environmental Protection Agency (EPA)

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and the Australian National Registration Authority, intensified in both countries by criticisms from representatives of public interest groups that the use of Bt-transgenic crops would put users of Bt sprays at risk of resistance. The area that can be planted to Bt-cotton in Australia remains restricted to less than 30% of the 300 000 ha crop due to concerns about resistance management (see Section 2.1). Resistance management has only rarely been a significant part of the registration process.[5]

Even after registration, resistance has continued to be a major issue in the USA. The current Bt-cotton cultivars, based on technology from Monsanto bred into varieties produced by its seed partners, including Delta and Pine Land (DeltaPine), provide excellent control of two of the three major Lepidopteran pests of cotton in the US, the pink bollworm *Pectinophora gossypiella* (Saunders) and tobacco budworm, *Heliothis virescens* (F.). These two insects have been the most problematic on cotton in the USA due to their frequent evolution of resistance to insecticides, and were targeted for a 'high dose/refuge' resistance management strategy (Section 2.1) on the Bt-cotton. Unfortunately, both the US and Australia also suffer from two other cotton bollworms, *Helicoverpa zea* (Boddie) and *Helicoverpa armigera* (Hübner), respectively, which are less sensitive to the available Cry toxins[6] and showed significant survival on Bt cotton crops in experimental trials as early as 1993.[7] Resistance has never been a problem for *H. zea*, presumably because of the wide range of alternative crops that are not sprayed. In contrast, *H. armigera* concentrates on cotton in the key regions in Australia, and has serious problems with resistance.[8,9]

When surviving *H. zea* were found on Bt-cotton on commercial farms in Texas (and other USA states), it was widely and incorrectly reported that the cotton was 'a failure', resulting in an 18·5% decline in the value of DeltaPine's stock in one day.[4] However, Bt-cotton still saved considerable insecticide applications and yield. Compared to historical averages of five to 12 insecticide sprays per year, Bt-cotton was treated zero to three times in the US.[10] In Australia, Bt-cotton in the 1996–97 growing season received an average of six fewer sprays than conventional cotton (B. Pyke, pers. commun.). It is now expected that the Bt cotton crop will expand to about one million hectares in the USA in 1997, and it would increase at least five-fold in Australia were it not for the current restrictions. Nonetheless, the survival of *H. zea* on cotton in the USA evoked calls from representatives of public interest groups that the 'high-dose' resistance management strategy had been a failure and that the registration of Bt-cotton should be revoked. (The US EPA has apparently declined to take any action.)

To fit into existing laws, Bt-transgenic varieties are referred to by the US EPA as 'plant pesticides'. From the standpoint of public perception, this is clearly a poor choice of words. Unfortunately, many growers, businessmen and researchers also seem to regard transgenic varieties essentially as a replacement for insecticide sprays.

However, transgenic crops can be far more than this. They offer truly novel approaches for the development of integrated pest management systems. First, because they eliminate or greatly reduce non-selective sprays for dominant pests, Bt-transgenic crops will allow increased reliance on biological control of secondary pests and, thereby, reduce pesticide use and improve prospects for reducing resistance problems in aphids, whiteflies, mites and so on. For example, Bt-transgenic potatoes eliminated the need for insecticide sprays for control of Colorado potato beetles (*Leptinotarsa decemlineata* (Say)) and thereby allowed predators (and probably parasitoids) to control aphids almost as effectively as systemic insecticides.[11] There is an increased abundance of non-target species in Bt-cotton compared to conventionally grown cotton[7] and, contrary to initial expectations, at least for the first year in Australia, there was no increase in problems with other pests including mites and plant bugs (B. Pyke, pers. commun.). Bt-transgenic maize in Texas could eliminate the need for sprays against Lepidopterous pests and greatly reduce sprays against spider mites. Second, due to their capacity to regulate the growth of pest populations, Bt-transgenic crops can facilitate the use of under-utilized management tactics such as crop rotation and pheromone disruption that are not generally sufficiently effective alone at moderate-to-high pest densities.[12]

2 RESISTANCE MANAGEMENT?

Given their advantages, Bt-transgenic crops would seem to have a bright future, but then there is the potential for resistance to Bt toxins in insects, as amply confirmed by resistance to Bt sprays in the diamondback moth, *Plutella xylostella* L.[13] Are Bt-transgenic crops to be considered nothing more than a pretty and environmentally friendly insecticide, to be replaced with others as soon as resistance evolves, or does the increased attention paid to resistance issues suggest that we could be on the threshold of a new era in resistance management?

Using currently developed technology, probably just four strategies can be plausibly considered for managing resistance to Bt-transgenic crops: (1) refuges of non-transgenic host plants in which susceptible insects can develop without exposure to Bt toxins, a strategy which is most effective where there is high toxin expression in the plants; (2) moderate expression of toxin such that some susceptible insects survive exposure; (3) different toxins deployed individually in different varieties in a mosaic in the same area; and (4) use of varieties where each plant has a mixture of toxins.[2,12–15]

2.1 Refuges (including high expression and seed mixes)

The impact of expression (in terms of mortality of insects exposed), initial resistance allele frequency and proportion of the population that escapes exposure can be investigated with simple simulation models.[2,12,14,16,17] Such simulations show that the most influential factor on the number of generations until the resistance alleles reach a high frequency is the mortality of heterozygotes, where an increase from 50% to greater than 95% can provide a 10-fold delay of resistance (Fig. 1). By contrast, even a 1000-fold difference in the initial frequency of the resistance allele generally makes less than a five-fold difference in the time to resistance (assuming that at least 80% of the population is under selection).

Refuges have long been observed to play a major role in delaying resistance,[18] but there is not a directly proportional benefit (e.g. a two-fold increase in refuge size does not give a two-fold delay of resistance) unless mortality of the heterozygotes approaches 90% (Fig. 1). Unfortunately, large refuges are not attractive in terms of maximising yields or minimising the use of insecticides. Companies marketing Bt-transgenic crops have generally been unwilling to support refuges of greater than 5% of the crop, unless these refuges are sprayed with insecticides, which reduces their capacity to produce significant numbers of susceptible insects and complicates efforts to manage resistance to both insecticides and transgenic crops (see Section 2.3). From these models, it is clear that the only way to support refuges of 5% or less is when mortality of heterozygotes is very high.

Initial allele frequency is difficult to estimate, but fortunately also appears to be the least influential on the time to resistance (Fig. 1). A recent estimate places the initial frequency of at least some major genes for Bt resistance at about 10^{-3} in *H. virescens*.[19] Not all readers of that paper[19] will be convinced that single genes were measured, but even a frequency of 10^{-3} is not critical so long as the mortality of heterozygotes is very high, in excess of 95% (Fig. 1). In the case of the genes studied in *H. virescens*, the Bt-cotton cultivars currently deployed killed 100% of heterozygous larvae,[19] and similar results have been obtained with Bt-resistant diamondback moths.[2,20] In such cases, a refuge as low as about 5% might provide a delay of resistance for at least 50 generations (Fig. 1). However, in the case of *Helicoverpa* species, where at least some fully susceptible larvae survive,[4,7,15] probably less than 95% of the heterozygotes are killed, which would require a much larger refuge, perhaps 50% of the population, to achieve the same delay of resistance.

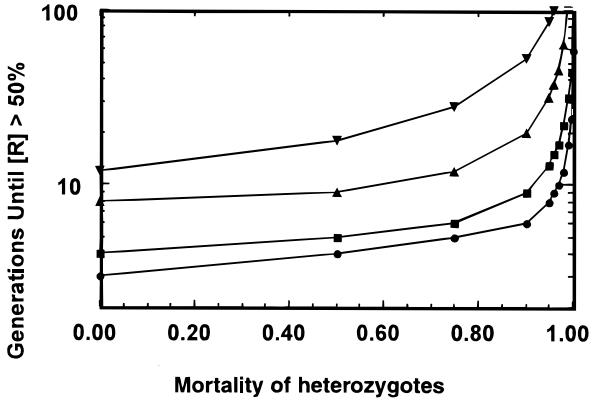

Fig. 1. Effect of mortality of RS heterozygotes and refuges on the evolution of resistance, as measured by the number of generations until the frequency of the resistance allele [R] exceeds 50% (note the log scale). Results of a simulation model assuming a single locus, random mating, no selective mortality of resistant homozygous larvae, that some fraction of the population escapes exposure (refuges of (●) 5, (■, ▲) 10 or (▼) 50%) and that the initial resistance allele frequency (p) is either (●, ■, ▼) 10^{-3} or (▲) 10^{-6}. Data points include 0, 50, 75, 90, 95, 96, 97, 98, 99, 99·5 and 100% mortality.

In the USA, 4% (not sprayed with insecticides for bollworms) or 20% (sprays acceptable) of the cotton crop is required by the US EPA to be non-transgenic as a refuge,[1] which is a strategy that assumes high mortality of heterozygotes in the more susceptible species, as has now been observed for some genes in *H. virescens*.[19] Because resistance has never been a serious problem in *H. zea* in the USA, it is assumed that much of the *H. zea* populations must be on non-cotton hosts, especially maize, i.e. the refuge is large. In contrast, resistance has been a serious problem in the closely related *H. armigera* in Australia, consistent with observations that *H. armigera* is closely associated with cotton. Population census and crop production data suggest that as much as 90–99% of the Australian *H. armigera* populations in some key areas develop on cotton, particularly in the late season (March, data from G. Fitt[9]), although in the middle of season, much of the population is on sorghum.[8,9] Thus, the introduction of Bt-cotton in Australia has been slowed by regulatory authorities (with support of researchers and cotton grower organisations) until a more sophisticated resistance management plan can be implemented. Under investigation is the intentional planting of highly attractive alternative host crops for those times of the year when non-cotton hosts are not abundant (such as late-planted and irrigated maize or sorghum). Already recommended is the suppression of overwintering pupae from Bt-cotton by specific ploughing practices (known locally as 'pupae busting', although all that is required is destruction of the pupal burrow so that the moths cannot reach the surface after eclosion)[9] and the use of narrow planting windows to reduce the number of generations per year under which selection can take place.

From an implementation standpoint, an attractive way of deploying refuges is as a seed mix such that there is ample opportunity for random mating of adults. Resistance management could essentially be sold 'in the bag' of seeds. In the absence of seed mixes, refuges are

generally meant to planted on the same farm within 1–2 kilometres of any transgenic plants. Unfortunately, seed mixes can be far worse for resistance than when the refuge is maintained outside the transgenic crop. When expression of the toxins is sufficiently high that mortality of heterozygotes exceeds 99% and more than about 20% of the larvae move from plant to plant, the addition of susceptible plants to the crop can allow increased proportions of heterozygotes to survive, wasting the benefits of the high expression.[14,16,17] For at least some of the pests of cotton and maize, pest larvae move too much between plants for the strategy to manage resistance effectively, and in some cases, to avoid damage to the crop.[12] In Australian cotton, for example, not only were more than 70% of *H. armigera* larvae observed to move between plants, but also many of them died once they got to a transgenic plant (a 25% seed mix that generated only 5% of the insects of a non-transgenic stand), and even transgenic plants suffered economically unacceptable feeding damage from larvae that grew large (and relatively insensitive to Bt) on susceptible plants (R. Roush and G. Fitt, unpublished).

With the introduction of Bt-transgenic maize also using similar Cry1A genes as in cotton,[21,22] the future of the refuge strategy for *H. zea* in the US is in doubt. Maize could now become a source of selection rather than a refuge.

2.2 Moderate expression

Bt-transgenic crops have unique characteristics of coverage and selectivity that potentially allow the effective use of a high dose. At pains to avoid a common source of confusion, I emphasise that there is no general advantage to applying more pesticide than is needed just to control the pest, and indeed high doses generally destroy the biological control agents in integrated management programs for insects and mites.[23,24] Perhaps the best experiments on dose have been conducted with fungicides, which have consistently failed to show an advantage with high application rates and, if anything, slightly slower resistance with lower rates.[25]

Using minimal doses of pesticide can allow more susceptible individuals to survive and thereby slow the development of resistance, although the benefits tend to be small. This can be illustrated using simulations of a single gene, such as with data for Bt resistance in the diamondback moth (Fig. 2). In addition to other problems, effectiveness of Bt at moderate doses can often be variable, which would at least be disheartening to growers if not wholly impractical.[12] For reasons still undetermined, efficacy in Bt-cotton in Australia in the 1996–97 growing season was highly variable, much more so than in previous years, with some of the transgenic cotton being sprayed almost as much as the non-transgenic cotton on the same farms (B. Pye, pers. commun.). Because the current Bt-cotton borders on a moderate dose for *Helicoverpa*, the effects of environmental influences on expression can be serious. Thus, although mortality levels around 80% seem unlikely to select for a single major resistance gene any faster than expression at 95% mortality, neither level has any significant advantage over aiming for high expression (Fig. 2). Where the technology exists to provide high levels of expression without yield loss,[22] varieties that allow some susceptible insects to survive[21] should be avoided.

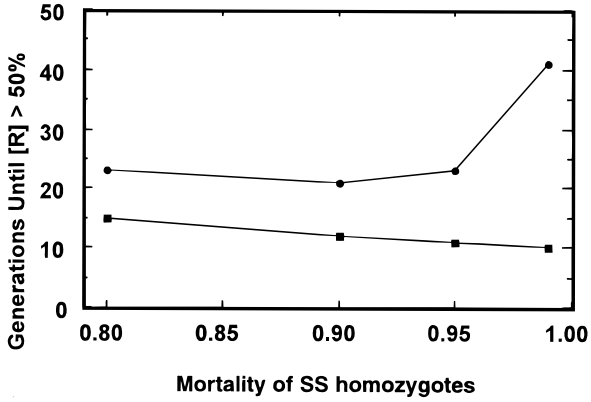

Fig. 2. Effects of low expression on the evolution of resistance. The initial resistance gene frequencies were 10^{-6}, and 20% of larvae escape exposure each generation. Results shown in terms of mortality of susceptible larvae; (●) 'Resistance Recessive' is based on resistance to Cry1A Bt toxins in the diamondback moth (where resistance is due to a single gene in at least some populations;[29] these simulations based on data from Tang *et al.*,[30] but similar results would be found with all other data cited by Tabashnik[13] on *P. xyllostella*). For example, where 80% of the susceptible homozygotes (SS) are killed, it was assumed that there was 50% mortality of heterozygotes (RS); at 95% mortality of SS there was 70% mortality of RS; at 99% mortality of SS there was 90% mortality of RS. (■) 'Resistance Dominant' assumes that heterozygotes are never killed by the transgenic crop.

2.3 Mosaics

Mosaics are the worst way to deploy two toxicants, potentially resulting in resistance about twice as fast as if they were used on different generations.[24,26] In spite of common agricultural practice (one farmer uses one pesticide while his neighbour uses another), avoiding mosaics with 'calendar-window based' rotational systems (e.g. as was done for *H. armigera* in Australia[8]) would probably be one of the simplest ways to improve the use of pesticides for resistance management. In contrast, the use of sprayed cotton as a refuge for transgenic cotton (Section 2.1) puts both the Bt-cotton and the insecticide at risk.

A more serious concern is with the development of Bt-transgenic maize, where competing seed companies may soon deploy varieties with either Cry1A[21,22] or Cry9C toxins, which do not apparently share binding sites or cross-resistance.[27] Because these toxins pri-

marily target the European corn borer, *Ostrinia nubilalis* (Hubner), a much more effective approach for resistance management would be to pyramid the genes.

2.4 Pyramided varieties

As described above, the main problems facing the introduction of Bt-crops include (1) providing a long delay of resistance, especially because the level of control of heterozygotes can be only guessed at until resistance evolves, (2) providing refuge sizes that are commercially acceptable and maximise the reduction of pesticide use and (3) avoiding selection of multiple toxins in mosaics. The most effective way to achieve these objectives is through pyramided varieties using two or more toxins. In contrast to the high expression strategy, it is the mortality of the susceptible homozygotes rather than heterozygotes that is a key to success, and one which can be more readily measured when developing the resistance management program.[2,12]

Assuming for the moment that there was no mechanism for cross-resistance between the two toxins or tight linkage between different resistance genes in the insects, pyramids can delay resistance more effectively on a smaller refuge size than the two toxins sequentially introduced. For example, a 5% refuge with a pyramid can delay resistance for about as long as a 30–40% refuge for the sequentially deployed toxins (Fig. 3). Based on a large number of simulation runs, it appears that a significant delay of resistance (greater than five-fold compared to the sequential introduction, not to mention a mosaic use, of two toxins) will occur roughly when the percentage refuge is greater than $h_A h_B(1 - r)$, where h_A and h_B are the survivals of heterozygotes resistant to toxins A and B respectively, and r is the recombination rate between the two resistance loci. Where it is assumed that the survival is 30% for heterozygotes for each of the two toxins when used alone, and that the two resistance genes are distantly linked or on separate chromosomes, the critical refuge size is (0·3) (0·3) (0·5) or 4·5% (Fig. 3).

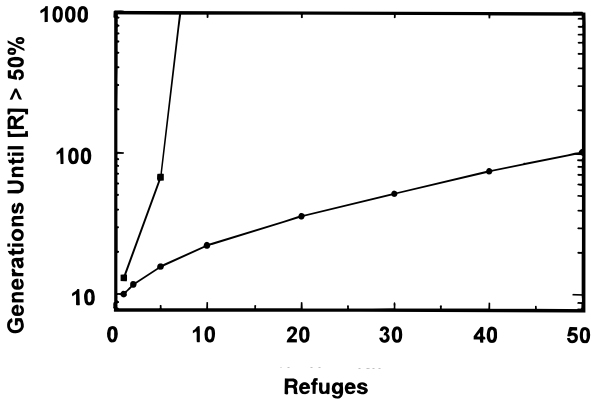

Fig. 3. The evolution of resistance with (●) sequential deployment of two toxins compared with (■) use of the toxins jointly in a pyramided variety, for a range of percentages of the population in refuges, as measured when the frequency of the resistance alleles [R] for both toxins exceeds 50% (note the log scale). For simplicity, it was assumed that the initial resistance allele frequency (p) was 10^{-6} for each toxin, there was 70% mortality of RS heterozygotes for both toxins, and 100% mortality of SS homozygotes for each toxin.

Chromosomal linkage of resistance genes will lower but not eliminate the benefits of pyramids.[28] Cross-resistance would also lower the benefits of pyramids, but simulations suggest that not much can be lost compared to sequential introduction of toxins, and less than with the mosaics of toxins. If genes for broad cross-resistance exist, they will also confer an advantage and be selected by even the first toxin gene used, at least until a more specific gene becomes common. Under the worst possible scenario, a pyramided crop would last only as long as a single toxin (i.e. half as long as introducing the two toxins sequentially), but a far more likely outcome, due to simultaneous selection for the cross-resistance gene, is that there would be very little difference between the pyramid and sequential introduction strategies. In short, there is little to lose and potentially a great deal to gain (e.g. Fig. 3).

3 CONCLUSIONS

There are some who have argued that the effects of Bt resistance in some crops such as maize would be minimal, and that we would merely return to the current status quo in terms of pest control. This view ignores the potential long-term benefits to pest management, not just in the crop concerned, but also the politics of the wider application of the technology. Public interest groups have already argued vigorously that Bt-crops should not be introduced because we cannot manage resistance. If we should prove them correct, it will surely strengthen their resolve and arguments to prevent the extension of Bt-transgenic technology to other markets and other pest-management problems, and even the suspension of registrations of products already commercialised. This is an issue where public sector entomologists and the private sector corporations have a common objective, even if for different reasons.

There are at least two commercial aspects of Bt-crops that are currently on a collision course with resistance management. First is the use of mosaics of different toxin types in maize in areas where corn borers are the major pest and cotton is not grown. The optimal solution would be to pyramid both the Cry1A and Cry9C genes. Second is the use of Cry1A in maize and cotton in the same areas, where both serve as hosts for *H. zea*. At present, the only effective way to address this problem would be to restrict the use of Cry1A to cotton and use Cry9C in maize where it will control most of the major pests but does not affect *H. zea*.[27]

As has always been true historically, the critical limiting factor for resistance management will be implementation. In the case of pyramiding strategies, the problem will most likely be conflicting commercial and intellectual property interests among the companies involved. Why should companies compromise? Even at an initial allele frequency of 10^{-3}, the pyramiding strategy may confer a 30- to 40-fold delay of resistance,[12] whereas resistance could otherwise evolve in as little as 10 generations, which could be three or four years for both corn borers and *H. zea* (Fig. 1). Obtaining these benefits would clearly seem to be in the interests of growers and the companies, but are perhaps too theoretical. There may be a more important justification: insurance. When resistance does evolve, the first varieties to fail will probably be those that have a single toxin, especially if it is the most widely used toxin, leaving those seed companies to scramble for the development of replacement lines, probably while losing market share. Pyramided lines will have a second toxin to protect them, at least for the short term. In sum, companies should consider pyramiding strictly out of self-interest.

If compromises can be reached among the companies involved, Bt-transgenic crops truly will have a chance to provide a dramatic new start in resistance management. If not, the worst fears of the critics of this technology may well be realised.

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